Inflammatory Bowel Disease Research Symposium, 2022

Autophagy differentially modulates Tight Junction pathways to reduce gut permeability and inflammation.

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I have no potential conflicts of interest to disclose.

Any names or ages used on the upcoming slides are fictitious and not referring to an actual patient.

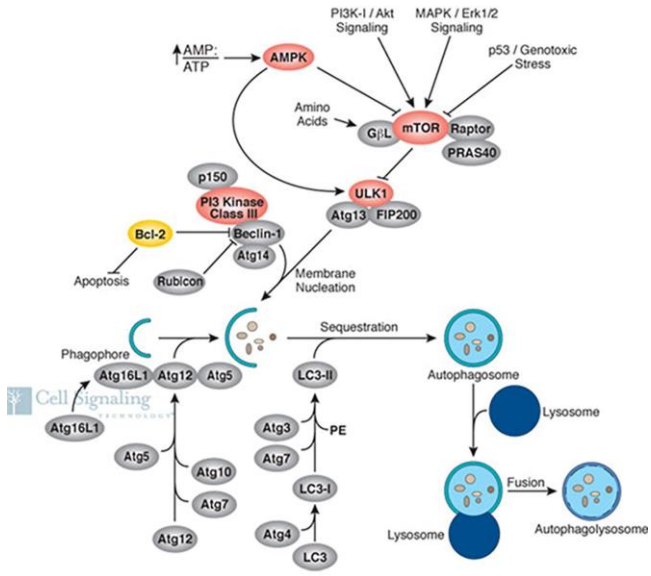
Is self-eating beneficial?

- **Autophagy** (*self-eating*) is a cell survival process that results in the autophagosomic-lysosomal degradation of cytoplasmic contents, abnormal protein aggregates, and excess or damaged organelles (Kaser and Blumberg, 2011).

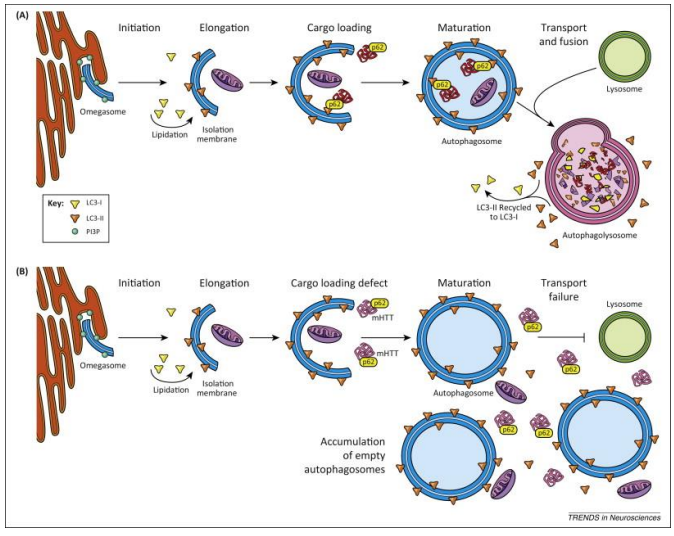
Outline:

- Review the autophagy process and its importance in intestinal function and Inflammatory Bowel Disease (IBD).
- Examine the research data on autophagy-mediated enhancement of intestinal Tight Junction barrier.

Autophagy Pathway



Autophagy is altered in Huntington disease



Trends in Neurosciences 2015 38, 26-35DOI: (10.1016/j.tins.2014.09.003)

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Autophagy: To be, or not to be

Autophagy inhibition

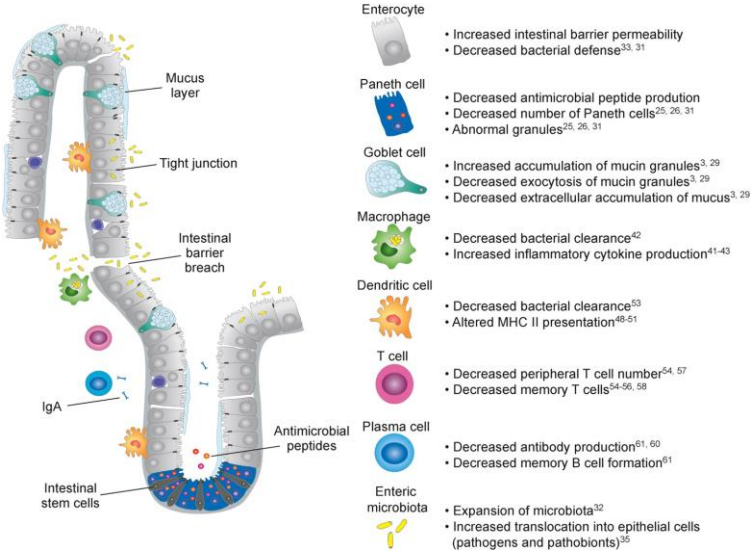
- Reduce fitness of cancer cells
- Metabolic alterations to reduce cancer growth
- Avoid autophagy-dependent cell death

Autophagy: To be, for sure

Autophagy induction:

- Age related diseases
  - Spermidine, Resveratrol
- Anti-cancer immunosurveillance
  - Recruitment of dendritic cells in tumor bed
- Cardiovascular diseases
  - Trehalose improves outcomes of myocardial infarction
- Infectious diseases
  - Mycobacterium clearance
- Neurodegeneration
  - Clearance of neurotoxic proteins
- Obesity
  - Chronic inhibition of autophagy accelerates aging diseases

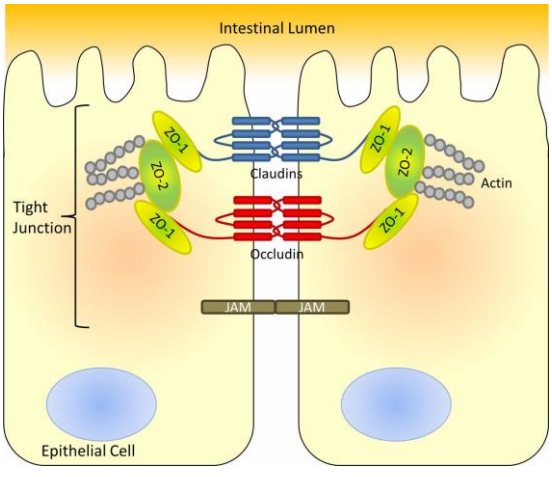
Defects in autophagy disturbs intestinal homeostasis



Baxt LA, Xavier RJ. Gastroenterology, 2015

Tight Junction barrier

Gate and Fence



Occludin: Barrier forming
Claudin-2: Pore forming

Fraser L. Collins et al. PHY2 2017;5:e13263

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Tight Junction Barrier defects in IBD

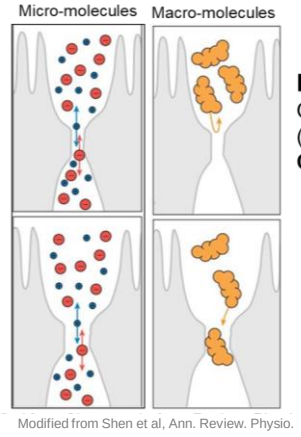
TJ morphology: abnormalities in the structural strands of the tight junctions in Crohn's disease (Marin et al., 1983).

TJ function: increased intestinal permeability in Crohn's disease patients (Hollander, 1988).

TJ composition:

Inflammatory bowel diseases	Pore forming TJ proteins	Barrier forming TJ proteins	References
Crohn's disease (sigmoid colon)	Claudin-2 ↑	Occludin ↓ Claudin-3 ↓ Claudin-5 ↓ and redistributed Claudin-8 ↓ and redistributed	Zeissig *et al.* 2007
Ulcerative colitis (sigmoid colon)	Claudin-2 ↑	Occludin ↓ Claudin-1 ↓ Claudin-4 ↓ Tricellulin ↓	Heller *et al.* 2005; Schmitz *et al.* 1999a; Krug *et al.* 2010
Collagenous colitis (sigmoid colon) (microscopic colitis)	Claudin-2 ↑	Claudin-4 ↓ Occludin ↓	Burgel *et al.* 2002; Tagkalidis *et al.* 2007

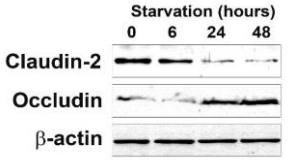
Autophagy targets TJ



Modified from Shen et al, Ann. Review. Physio.

Pore Pathway
Charge/ size selective
(cations/ < 4 Å)
Claudin-2

Leak Pathway
non-charge-selective,
large-size
Occludin



(Nighot et al., JBC, 2015)

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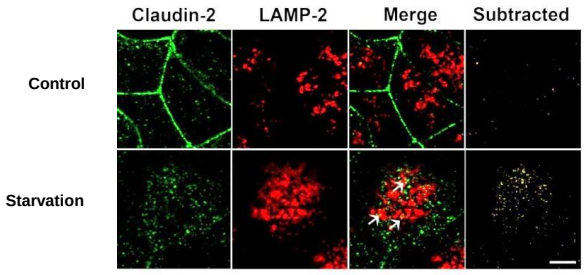
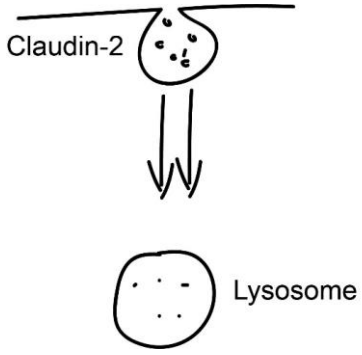
Autophagy enhances TJ barrier via degradation of claudin-2

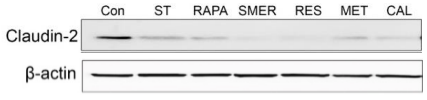


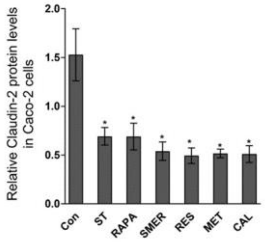


(Nighot et al., JBC, 2015)

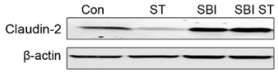
Autophagy mediated reduction in claudin-2 levels

Multiple autophagy inducers degrade claudin-2

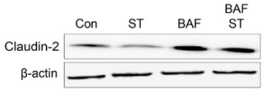


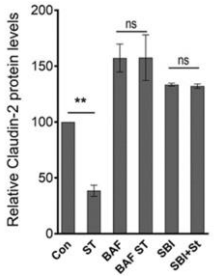


Inhibition of autophagy initiation

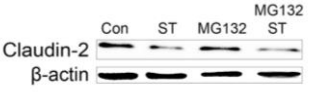


Inhibition of autophagosome-lysosome fusion

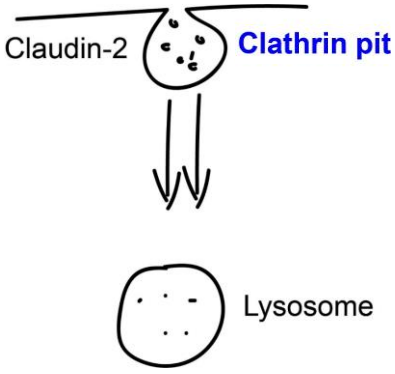


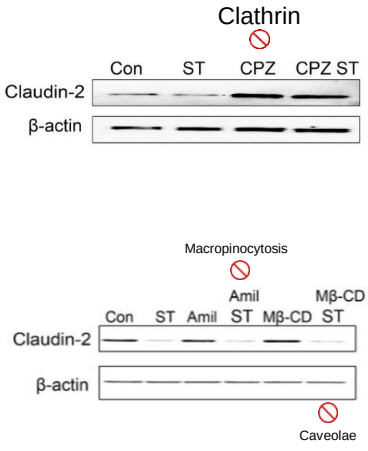


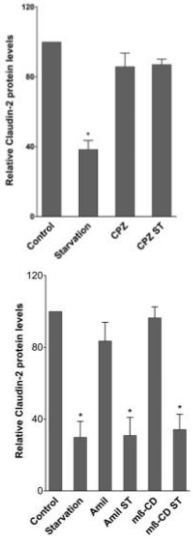
No proteasomal degradation



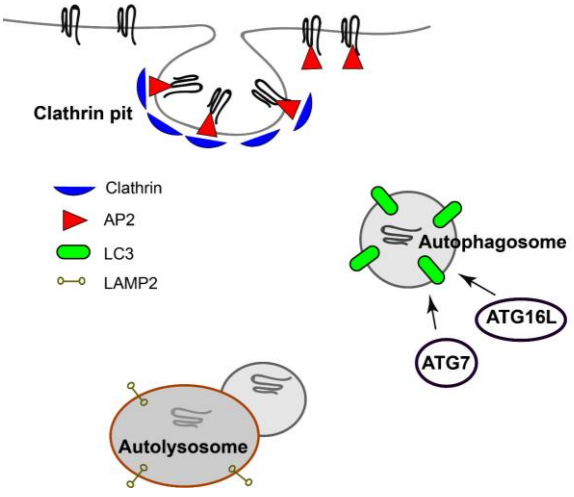
Autophagy-induced claudin-2 reduction is mediated via clathrin pits

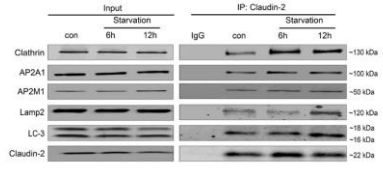


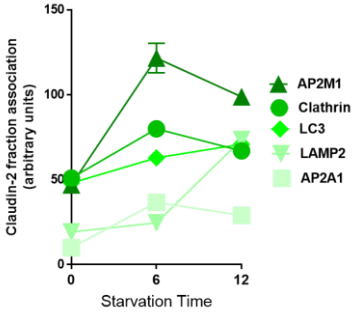




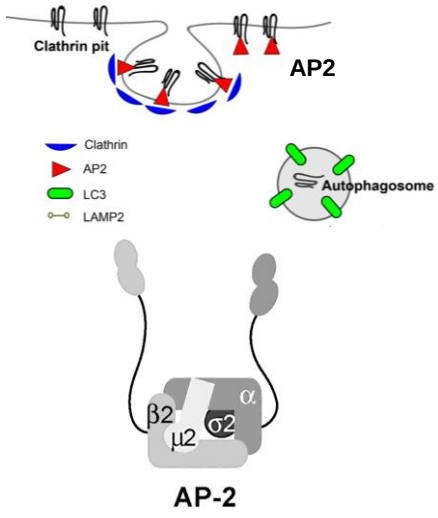
Endocytosis-Autophagy intersection

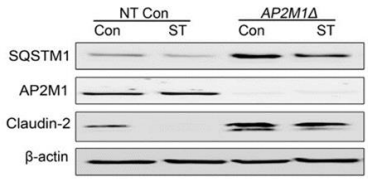


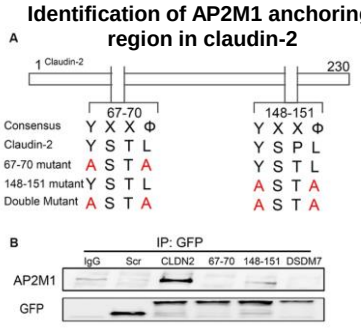




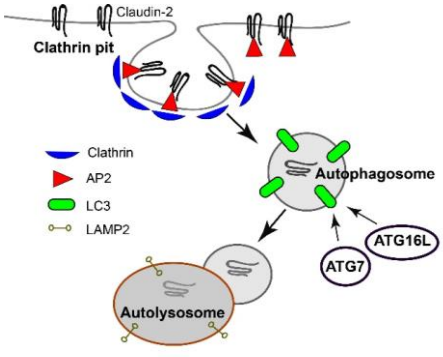
AP2M1 is required for autophagy-mediated reduction in claudin-2

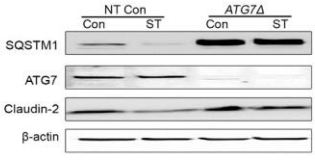


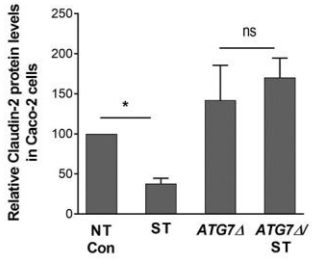




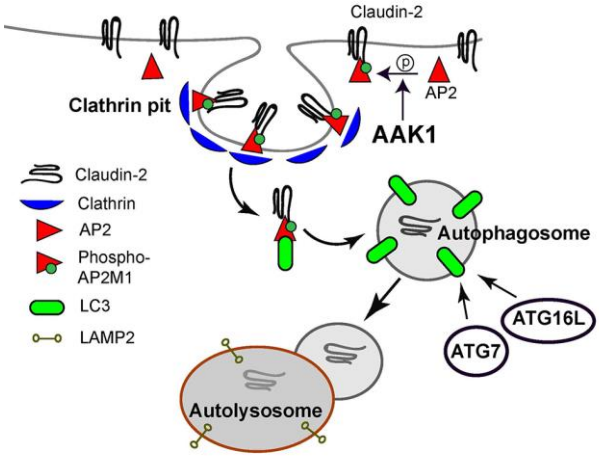
Intersection of claudin-2 endocytosis and autophagy







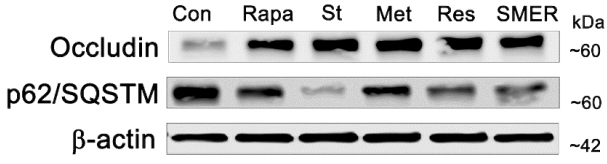
Summary: Autophagy degrades claudin-2.



- Autophagy promotes clathrin-mediated claudin-2 endocytosis; Adaptor protein AP2M1 subunit plays a crucial role in this process.
- AP2M1 binds to claudin-2 and also interacts with LC3 bringing claudin-2 to autophagy pathway.

Ganapathy et al., *Autophagy, 2022*

Novel role of Autophagy in increasing cellular occludin levels



Con: Control; **Rapa**: Rapamycin; **St**: Starvation; **Met**: Metformin; **Res**: Resveratrol; ; **SMER**: SMER-28

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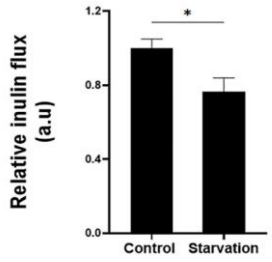
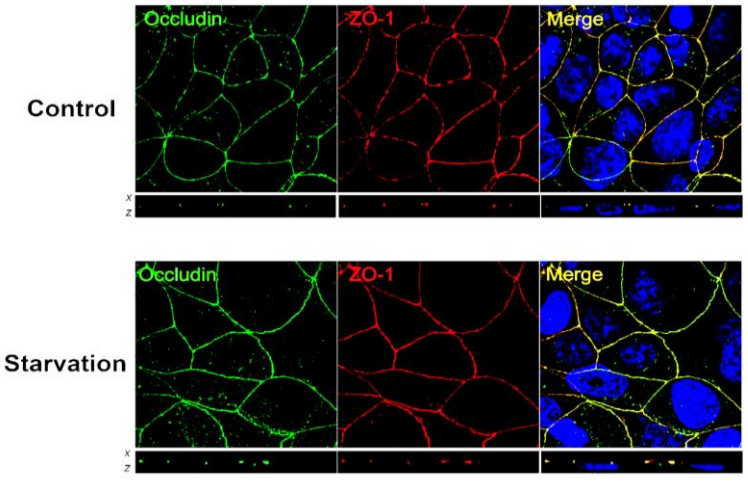
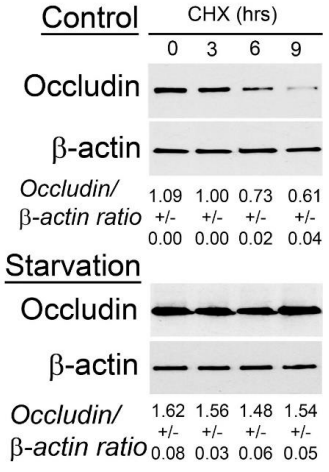
Autophagy enhances membrane localization of occludin and the TJ barrier



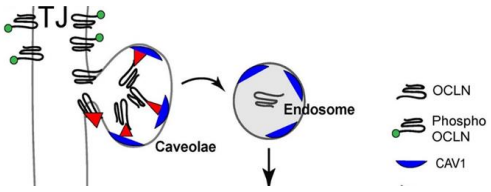


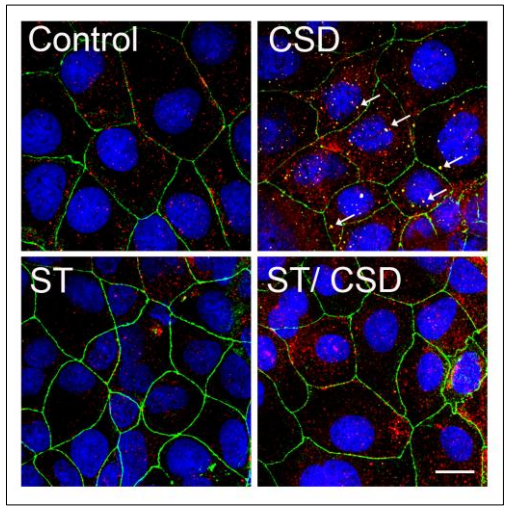
Autophagy protects occludin from degradation



CHX: Cycloheximide; **ST**: Starvation; **CSD**: **C**aveolin-1 **S**caffold **D**omain mimicking peptide.

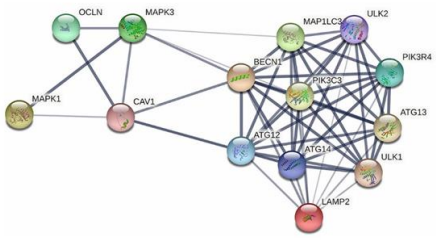
Autophagy protects occludin from degradation

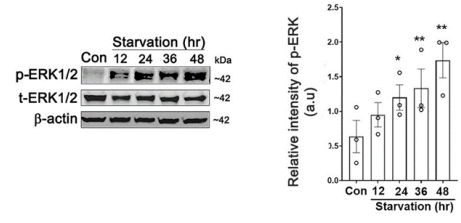


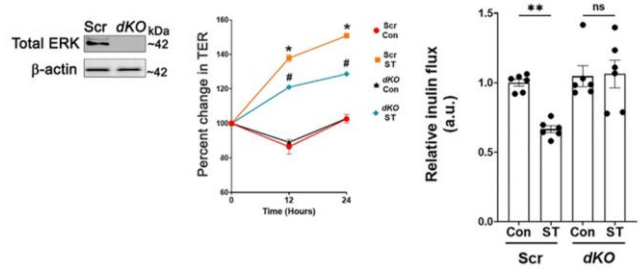


White bar = 5µm

Autophagy upregulates occludin via ERK1/2







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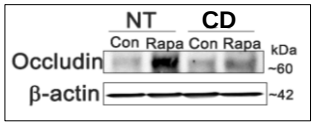
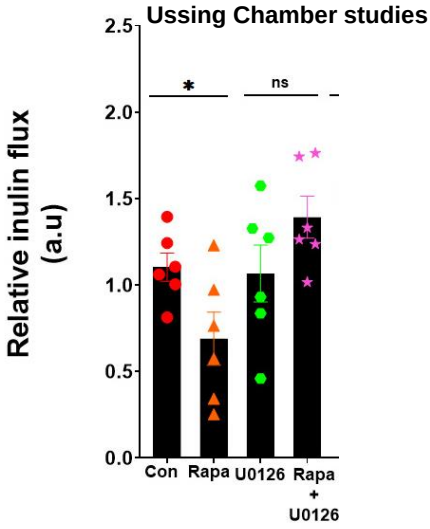
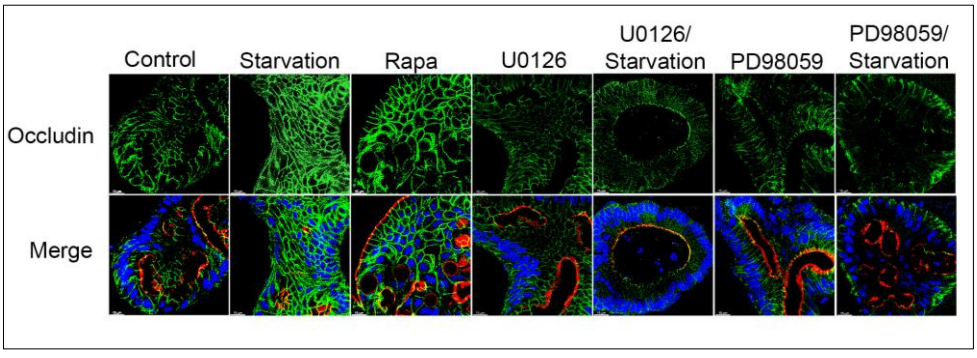
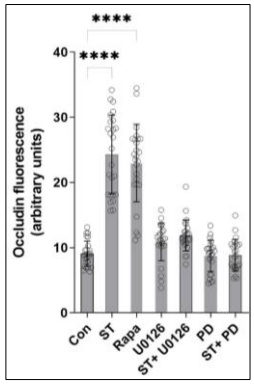
Autophagy enhances occludin levels and the TJ barrier in human colonic tissue





Autophagy enhances occludin levels in human colonoids





Occludin F-actin Nucleus

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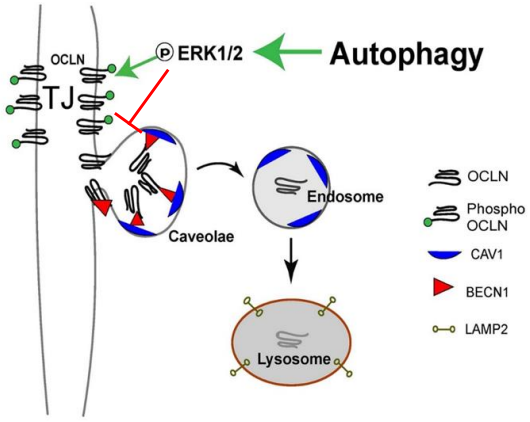
Summary: Autophagy enhances TJ barrier by increasing occludin levels



- Autophagy enhances the intestinal TJ barrier by upregulating levels of the barrier-forming protein occludin.
- The ERK1/2 kinases protect occludin from degradation upon autophagy induction.

Saha et al., *J of Crohn's and Colitis, 2022*

In vivo studies

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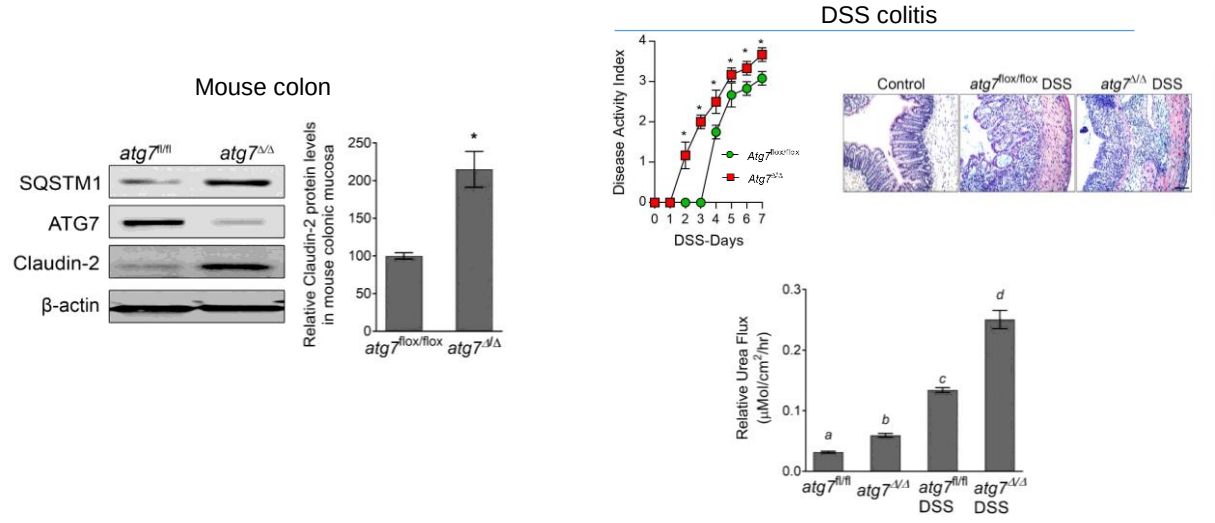
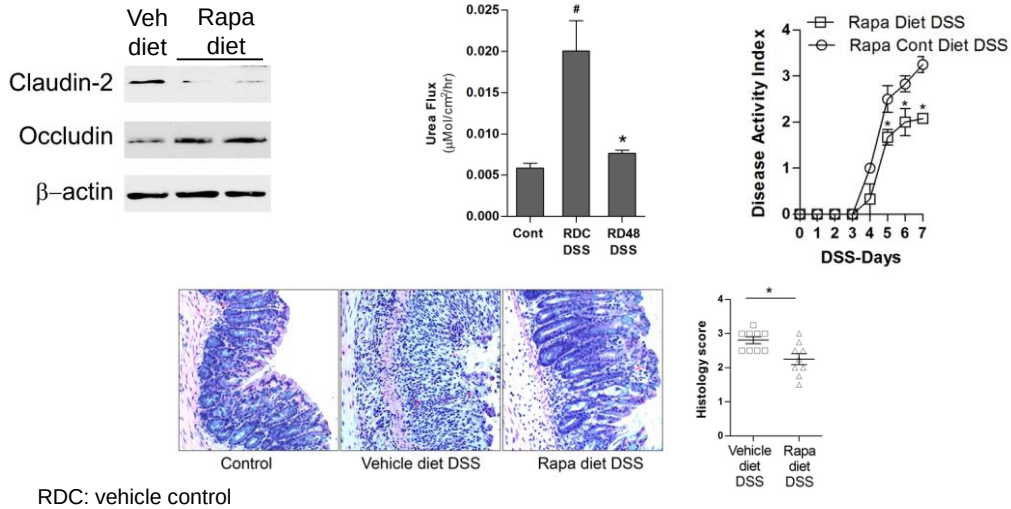
Atg7 deletion compromises mouse TJ barrier



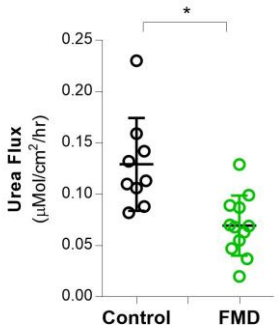
(Ubc-Cre-Ert2-Atg7fl/fl mice treated with Tamoxifen)

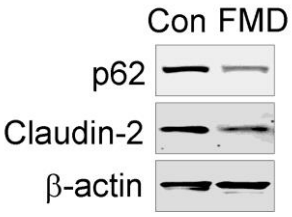
eRapa diet attenuates DSS-induced increase in colonic permeability



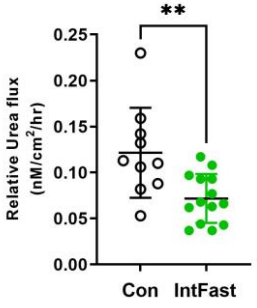
RDC: vehicle control
RD: eRapa diet

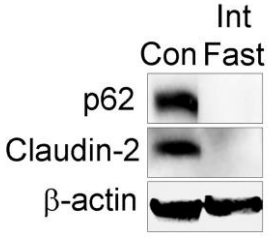
Fasting Mimicking Diet (FMD) induces autophagy and reduces TJ permeability





Intermittent Fasting (IntFast) induces autophagy and reduces TJ permeability





8-hrs feeding, 16-hrs fasting x 7 days

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Autophagy outcome

Substrate	Cell type	Extent	Context	Autophagy-independent function of ATG proteins
Claudin-2/ Occludin	Epithelial vs Non-epithelial	Too much: Cyto-destruction	Homeostasis vs disease	E.g. ATG6/ beclin-1 regulates occludin

Summary:

Autophagy reduces gut permeability via reduction in pore forming claudin-2 and increase in barrier forming occludin levels.

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  NIH/ NIGMS

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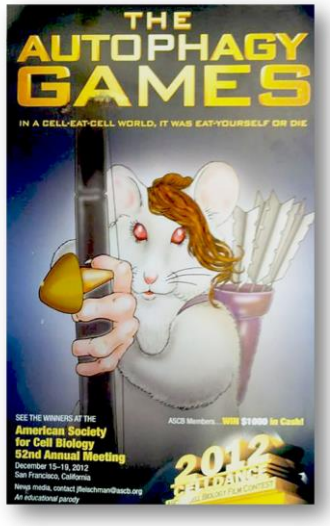
Thank You



Any names or ages used on the upcoming slides are fictitious and not referring to an actual patient.